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EXAMINER	
FOSTER, CHRISTINE E	

ART UNIT	PAPER NUMBER
1641	

DATE MAILED: 07/17/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/620,332	VOYTA ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-10,13-19 and 21-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-10,13-19 and 21-45* is/are rejected.

7)☒ Claim(s) *6,33,40 and 42* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/9/06 has been entered.

Claims 1 and 40-43 have been amended. Claims 2 and 11-12 have been canceled.

Claims 1, 3-10, 13-19, and 21-45 are currently pending and under examination.

Objections/Rejections Withdrawn

2. The Examiner's statement regarding proper amendment format (see the previous Office action at p. 2) was in error, as noted by Applicant (Applicant's response, p. 10). The amendment filed 11/1/05 correctly included the text claim 1.

3. The rejection of claim 1 under 35 USC 112, 1st paragraph (new matter) is withdrawn in response to Applicant's arguments (p. 10-11).

4. The rejections of claims 10-12 under 35 USC 112, 1st paragraph (enablement) have been withdrawn in response to Applicant's arguments (p. 12), upon further consideration by the Examiner, and in light of the cancellation of claims 11-12.

5. The rejections under 35 USC 112, 2nd paragraph not reiterated below have been withdrawn.

Priority

6. The first paragraph of the specification identifies the instant application as being "related" to copending applications 10/046,730 and 10/462,742, and to application 10/050,188 (now US 6,905,826). If this reference is intended as a claim for priority under 35 USC 120, please see the remarks following. If the reference is not intended as a claim for priority, no action is required.

If applicant desires to claim the benefit of a prior-filed application under 35 U.S.C. 120, a specific reference to the prior-filed application in compliance with 37 CFR 1.78(a) must be included in the first sentence(s) of the specification following the title or in an application data

sheet. **For benefit claims under 35 U.S.C. 120, 121 or 365(c), the reference must include the relationship (i.e., continuation, divisional, or continuation-in-part) of the applications.**

If the instant application is a utility or plant application filed under 35 U.S.C. 111(a) on or after November 29, 2000, the specific reference must be submitted during the pendency of the application and within the later of four months from the actual filing date of the application or sixteen months from the filing date of the prior application. If the application is a utility or plant application which entered the national stage from an international application filed on or after November 29, 2000, after compliance with 35 U.S.C. 371, the specific reference must be submitted during the pendency of the application and within the later of four months from the date on which the national stage commenced under 35 U.S.C. 371(b) or (f) or sixteen months from the filing date of the prior application. See 37 CFR 1.78(a)(2)(ii) and (a)(5)(ii). This time period is not extendable and a failure to submit the reference required by 35 U.S.C. 119(e) and/or 120, where applicable, within this time period is considered a waiver of any benefit of such prior application(s) under 35 U.S.C. 119(e), 120, 121 and 365(c). A benefit claim filed after the required time period may be accepted if it is accompanied by a grantable petition to accept an unintentionally delayed benefit claim under 35 U.S.C. 119(e), 120, 121 and 365(c). The petition must be accompanied by (1) the reference required by 35 U.S.C. 120 or 119(e) and 37 CFR 1.78(a)(2) or (a)(5) to the prior application (unless previously submitted), (2) a surcharge under 37 CFR 1.17(t), and (3) a statement that the entire delay between the date the claim was due under 37 CFR 1.78(a)(2) or (a)(5) and the date the claim was filed was unintentional. The Director may require additional information where there is a question whether the delay was

unintentional. The petition should be addressed to: Mail Stop Petition, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

If the reference to the prior application was previously submitted within the time period set forth in 37 CFR 1.78(a), but not in the first sentence(s) of the specification or an application data sheet (ADS) as required by 37 CFR 1.78(a) (e.g., if the reference was submitted in an oath or declaration or the application transmittal letter), and the information concerning the benefit claim was recognized by the Office as shown by its inclusion on the first filing receipt, the petition under 37 CFR 1.78(a) and the surcharge under 37 CFR 1.17(t) are not required. Applicant is still required to submit the reference in compliance with 37 CFR 1.78(a) by filing an amendment to the first sentence(s) of the specification or an ADS. See MPEP § 201.11.

Claim Objections

7. Claims 6, 33, 40 and 42 are objected to because of the following informalities:

Claims 6 and 33 are objected to because they appear to recite subject matter of identical scope.

In claim 40 it appears that the word "salts" should be place outside of the parentheses in the recited onium polymers.

In claim 42 it is suggested that the abbreviation "BSA" be accompanied by the full term.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claims 24 and 30 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

10. Claims 24 and 30 recite that fluorescent labels are attached directly or indirectly to the surface layer. The specification discloses that the support surface may comprise fluorescent labels (p. 9, lines 11-18), but support cannot be found for fluorescent labels attached "directly or indirectly" to the surface layer. Applicant indicated that support may be found in the specification at p. 15, lines 23-24 and at p. 16, lines 9-11. The specification discloses that control labels (which include fluorescent labels as disclosed at p. 16, lines 9-11) can be attached "directly to a probe for a target molecule or via attachment to a different molecule" (p. 15, line 17 to p. 16, line 11). The specification also discloses in this section that a control label can be attached to a control target capable of binding to a control probe. Although this latter would be considered to be an example of "indirect" attachment, the disclosure of these two specific examples of "direct" and "indirect" attachment do not provide support for the genus of direct or indirect attachment of fluorescent labels that is now being claimed.

Applicant is effectively claiming a subgenus not supported by the specification as filed. The disclosure of a specific example of "direct" attachment (control labels attached directly to probes for a target molecule) does not support the subgenus of fluorescent labels attached directly to the surface layer as claimed. Such a subgenus would also include direct attachment of

the fluorescent label to the surface (not via a probe), for which no support could be found in the specification. Likewise, the disclosure an example of "indirect" attachment (control probes attached to control targets that are capable of binding to a control probe) does not support the subgenus of fluorescent probes indirectly attached to the surface layer. It cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. See In re Smith 173 USPQ 679, 683 (CCPA 1972) and MPEP 2163.05.

11. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

12. Claims 1, 3-10, 13-19, and 21-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

13. Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential structural cooperative relationships of elements, such omission amounting to a gap between the necessary structural connections. See MPEP § 2172.01. The omitted structural cooperative relationships are: that the probes are **immobilized** on the surface layer of the solid support. The claim recites that a plurality of probes "disposed in" discrete areas on the surface layer of the solid support. The specification discloses that microarray technology involves that the probes be *immobilized* in discrete areas (p. 1, line 21). It would seem to be essential that the probes are immobilized in order to detect different signals in different discrete areas, since otherwise, the probes could be washed away upon application of the liquid substrate

composition. It is suggested that the claim recite that the plurality of probes are "immobilized" rather than "located" in the plurality of discrete areas.

14. Claim 13 recites the limitation "the support surface" in line 4. There is insufficient antecedent basis for this limitation in the claim.

15. Claims 13-19, 29-31, and 37-38 are indefinite for the following reasons. Claim 13 recites that the surface layer of the solid support is contacted with a sample that comprises the first and second target molecules. Claim 14 recites that the first and second target molecules are labeled with the first and second enzymes to form the first and second enzyme conjugates, respectively. These claims (and the claims that depend from them) therefore appear to describe a step in which the first and second enzyme conjugates become bound to the solid support. However, the claims depend from claim 1, which recites that the first and second enzyme conjugates are already bound to the surface layer. It is unclear how the enzyme conjugates may be contacted with the surface layer when they are already bound to the surface layer.

16. Claim 19 recites the limitations "the concentration" and "the expression level". There is insufficient antecedent basis for these limitations in the claim.

17. Claims 26 and 30 recite the limitation "the intensity". There is insufficient antecedent basis for these limitations in the claims.

18. Claim 31 recites "contacting the surface layer with **the sample comprising the first target molecule**". There is insufficient antecedent basis for this limitation since claim 13 refers to a "sample comprising first target molecules...**and second target molecules**".

19. Claims 42-43 recite the limitation "the composition comprising the chemiluminescent quantum yield enhancing material". There is insufficient antecedent basis for this limitation since

claim 1 only refers to a "chemiluminescent quantum yield enhancing material" and not to a composition.

20. Claim 44 recites the limitation "the corresponding target molecules". There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 103

21. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

22. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

23. Claims 1, 3-5, 9, 13-15, 21, 29, 31-32, and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. ("Chemiluminescence Detection for Hybridization Assays on the Flow-Thru Chip, a Three-Dimensional Microchannel Biochip," *Anal. Chem.* **73**:5777-5783 (2001)) in view of in view of Akhavan-Tafti (US Patent No. 6,068,979).

Cheek et al. teach a method of sequentially detecting chemiluminescent emissions on a solid support (microchannel glass) that includes the steps of contacting a surface layer of the solid support with a composition comprising a first chemiluminescent substrate (the luminol-based substrate Super Signal West Femto Maximum Sensitivity Substrate) capable of being activated by a first enzyme (horseradish peroxidase) to produce a first chemiluminescent signal; detecting the first chemiluminescent signal (see p. 5780, left column, "Detection," and pp. 5781-5782, "Two-Channel Chemiluminescence"); contacting the surface layer of the solid support with a composition comprising a second chemiluminescent substrate (the acridan phosphate-based substrate APS-5) capable of being activated by a second enzyme (alkaline phosphatase) to produce a second chemiluminescent signal; detecting the second chemiluminescent signal; wherein a plurality of probes are disposed in a plurality of discrete areas on the surface layer (see p. 5778, lines 2-14; p. 5779, left column, "Chip Preparation," lines 1-4 and right column, lines 11-12). Cheek et al. teach arrays on microchannel glass (and on flat glass) spotted with 500 micron center-to-center spacing (p. 5780, "Signal Intensity and Uniformity). This means that the discrete areas (spots) were separated by 0.05 cm, giving a spot density of 1 spot per 0.05 cm or 400 spots per cm^2.

The first and second enzyme conjugates are each bound indirectly to a probe (biotin-labeled target molecules are bound to streptavidin-HRP conjugates via biotin-streptavidin interaction and FITC-labeled targets are bound to anti-fluorescein-alkaline phosphatase conjugates via FITC/anti-fluorescein interaction). The labeled nucleic acids are contacted with the support surface prior to addition of the substrate composition (see Table 1 and pp. 5781-5782, "Two-Channel Chemiluminescence"). Detection and quantification of target molecule

binding to discrete areas of the chip is through image analysis (p. 5782, left column and Figure 5). The solid support is washed before contact with the first and second substrate compositions (p. 5779, "Hybridization assay," and p. 5782, left column). The enzyme conjugates were bound to the probes prior to contacting the surface layer of the solid support with the compositions comprising the chemiluminescent substrates (see p. 5781-5782, "Two-Channel Chemiluminescence" and p. 5779-5780, "Hybridization Assay" and "Detection").

Cheek et al. fail to specifically recite that the chemiluminescent substrate compositions are contacted with the support in the presence of a quantum yield enhancing material.

Akhavan-Tafti '979 teaches a method for sequential detection of multiple analytes by chemiluminescent emission on a solid support (the abstract), wherein surfactant enhancers are used to improve the signal/background ratio of enzymatically-produced chemiluminescence. Akhavan-Tafti '979 further teaches that suitable surfactant enhancers are known in the art and include polymeric onium salts, including quaternary phosphonium salts and ammonium salts, monomeric quaternary phosphonium and ammonium salts such as cetyltrimethylammonium bromide and dicationic surfactants (column 10, lines 29-47).

Therefore, it would have been obvious to one of ordinary skill in the art to employ surfactant enhancers, as taught by Akhavan-Tafti '979, in the method of Cheek et al. in order to improve the chemiluminescent signal/background ratio. One would have a reasonable expectation of success because Akhavan-Tafti '979 teach the enhancers for use in methods for sequential detection of enzymatically-produced chemiluminescence, which also describes the method of Cheek et al.

With respect to claims 5 and 44, Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Therefore, it would also have been obvious to one of ordinary skill in the art at the time of the invention to substitute the streptavidin-HRP conjugate of Cheek et al. with an antidigoxigenin-HRP conjugate for detection of a second target molecule labeled with digoxigenin, because Akhavan-Tafti '979 teaches that both antigen-antibody and biotin-streptavidin are both examples of binding pairs that may be successfully used for the purpose of acting as binding pairs in a method of sequential chemiluminescent detection of multiple, differentially labeled target molecules, such as that of Cheek et al.

24. Claims 1, 3-5, 7, 9, 13-15, 21, 25, 27-29, 31-32, 40-41, and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. ("Chemiluminescent Detection of DNA in Low- and Medium-Density Arrays," *Clinical Chemistry* **44**:2065-2066 (1998)).

Akhavan-Tafti '979 teaches a method of detecting chemiluminescent emissions on a solid support substantially as claimed. The method includes the steps of contacting a surface layer of the solid support with a composition comprising a first chemiluminescent substrate capable of

being activated by a first enzyme to produce a first chemiluminescent signal, detecting the first chemiluminescent signal on the surface layer of the solid support, contacting the surface layer of the solid support with a composition comprising a second chemiluminescent substrate capable of being activated by a second enzyme to produce a second chemiluminescent signal, and detecting the second chemiluminescent signal on the surface layer of the solid support (see in particular column 6, lines 17-24, 41-45 and 60-67; and column 7, lines 1-46; column 10, lines 59-67; and column 11, lines 1-44).

Akhavan-Tafti '979 fails to specifically teach a method wherein a plurality of probes is disposed on the surface layer at a density of at least 50 or at least 100 discrete areas per cm^2.

However, Akhavan-Tafti et al. teach chemiluminescent detection of DNA in low- and medium-density arrays of 100 spots per cm^2 (p. 2065, right column, paragraph 4). Akhavan-Tafti et al. further teach that such arrays are useful in high-throughput analysis of gene mutations and gene expression (p. 2065, right column, paragraph 1) and can be combined with chemiluminescent analysis with no expensive instrumentation (p. 2066, right column, last paragraph).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ the method of sequentially detecting chemiluminescent emissions of Akhavan-Tafti '979 using arrays with a plurality of probes disposed on a surface layer at a density of at least 50 or 100 discrete areas per cm^2 as taught by Akhavan-Tafti et al. in order to allow high-throughput analysis of gene mutations and expression. One would have had reasonable expectation of success in combining the array of Akhavan-Tafti et al. with the sequential chemiluminescent detection method of Akhavan-Tafti '979 because Akhavan-Tafti et

al. established that chemiluminescent detection was feasible with array formats. One would also have a reasonable expectation of success because the sequential detection method of Akhavan-Tafti '979 involves detection of chemiluminescent emissions on a nylon membrane, which is the same material used as the solid support in Akhavan-Tafti '979.

With respect to claims 38, 31, and 40-41, Akhavan-Tafti '979 also discloses use of chemiluminescent quantum yield enhancing materials, which may be onium polymers (column 10, lines 24-47) including poly(vinylbenzylammonium salts) and which may be present in the chemiluminescent substrate composition (see column 9, lines 46-52; column 10, lines 15-47 and US Patent 5,45,347, which was incorporated by reference). Chemiluminescent substrates are employed as buffered compositions (column 9, lines 46-47; column 10, lines 42-45, and column 15, lines 1-8). Washing of the solid support may be performed prior to contacting with the first substrate composition (column 15, lines 48-51) or after the first detection step (column 13, lines 9-13).

With respect to claims 3-5, 7, and 44, Akhavan-Tafti '979 teaches first and second enzyme conjugates that are bound directly to probes or are bound to first and second target molecules that are bound to probes (columns 15-16, Example 2; column 14, lines 29-33 and 60-67; column 4, lines 31-40; column 5, lines 18-24). Also disclosed are antidigoxigenin:enzyme conjugates wherein the corresponding target molecules are labeled with digoxigenin (columns 15-16, Example 2). The enzyme conjugates are added prior to the addition of the substrate compositions (see column 15, lines 40-45 for example).

With respect to claims 25 and 27, Akhavan-Tafti '979 teach a variety of first and second substrates, including Lumigen PS-3 and Lumi-Phos Plus, which have emission maxima of 430

and 470 nm, respectively. Because the instant specification and claims do not define what wavelengths or range of wavelengths constitute "approximately the same" emission maxima, the examiner has considered that the emission maxima of 430 and 470 nm also meet this limitation.

Therefore, it would have been clearly obvious to employ the substrates luminol and APS-5, which have "approximately" the same but different emission maxima as evidenced by Weimar et al. in the method of Cheek et al. and Akhavan-Tafti et al. since these are the specific substrates are taught in Cheek et al.

Akhavan-Tafti '979 also discloses contacting a support surface with a sample comprising first and second target molecules labeled with a first second label (e.g., lambda phage DNA labeled with biotin and SPPI marker DNA labeled with digoxigenin) prior to contacting the support surface with the substrate composition (columns 15-16, Example 2 in particular). With regard to claim 31, the surface layer may be washed after contact with the first target molecule and prior to contact with the first chemiluminescent substrate (see column 15, lines 48-51). Target molecules can include pools of target nucleic acids and mRNA for expression studies (column 14, lines 12-17) and may be quantified (column 1, lines 55-58).

25. Claims 25 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti et al. as applied to claim 1 above, and as evidenced by Weimer et al. (US Patent Application Publication 2004/0009529 A1).

Cheek et al. are Akhavan-Tafti '979 are as discussed above. Cheek et al. fails to specifically recite that the luminol and acridan phosphate-based substrates employed have "approximately" the same or different emission maxima.

Weimer et al. is relied upon as an evidentiary reference teaching that luminol emits light at a maximum of 425 nm, and that APS-5 emits light at a maximum of 430 nm (paragraph 66). Therefore, the luminol and acridan phosphate-based substrate APS-5 used in Cheek et al. have different emission maxima. With respect to claim 27, because the instant specification and claims do not define what wavelengths or range of wavelengths constitute "approximately the same" emission maxima, the examiner has considered that the emission maxima of 425 and 430 nm could fulfill this limitation as well, thereby meeting the limitation of claim 27.

Therefore, it would have been clearly obvious to employ the substrates luminol and APS-5, which have "approximately" the same but different emission maxima as evidenced by Weimar et al. in the method of Cheek et al. and Akhavan-Tafti et al. since these are the specific substrates are taught in Cheek et al.

26. Claim 28 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti et al. as applied to claim 1 above, and further in view of Giri et al. (US 6,602,679 B2).

Cheek et al. and Akhavan-Tafti et al. are as discussed above. Cheek et al. teach first and second chemiluminescent substrates (luminol and the acridan substrate APS-5), but fail to specifically teach that the substrates are provided as buffered compositions.

Giri et al. teach stable chemiluminescent formulations for use as substrates having extended shelf life, in particular formulations including suitable buffers in order to bring the substrate within the useful pH range (see the abstract; column 4, line 56 to column 6, line 64).

Therefore, it would have been obvious to one of ordinary skill in the art to provide the chemiluminescent substrates of Cheek et al. as buffered compositions as taught by Giri et al. in order to bring the substrate within useful pH range.

27. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Huang ("Detection of multiple proteins in an antibody-based protein microarray system," Journal of Immunological Methods **255**:1-13 (2001)).

Cheek et al. fail to teach a method wherein the first and second enzyme conjugates are each bound directly to probes.

Huang teaches detection of multiple proteins in a protein microarray using enhanced chemiluminescence detection. In one embodiment, immunoglobulin probes were spotted onto a membrane and detected by incubation with anti-immunoglobulin antibody probes that were bound directly ("conjugated") to HRP (see p. 4-6, section 3.1 and Table 2). Huang teaches that detection of the HRP targets binding to immobilized immunoglobulins was useful in testing the specificity and sensitivity of the assay (p. 6, left column, paragraphs 2-4).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ enzyme conjugates bound directly to probes, as taught by Huang et al., in order to detect immunoglobulin and/or to assess the specificity and sensitivity of an assay involving a protein microarray with chemiluminescent detection, such as that of Cheek et al.

28. Claims 8 and 40-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 as applied to claim 1 above, and further in view of Bronstein et al. (US Patent No. 6,602,658 B1).

Cheek et al. and Akhavan-Tafti '979 are as discussed above, which fail to teach specifically a method wherein the surface layer is contacted with quantum yield enhancing material before contacting the surface layer with the composition comprising the first chemiluminescent substrate, or where the quantum yield enhancing material is an onium polymer listed in claim 40, an onium copolymer, or where the quantum yield enhancing material further comprises an additive selected from the group listed in claim 42.

Bronstein et al. teach a method of measuring gene activity using sequential chemiluminescent detection of signal from two or more chemiluminescent substrates, wherein chemiluminescent signal enhancers such as polyvinylbenzyltrimethylammonium chloride (TMQ), onium copolymers, or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide the quantum yield enhancing material before contacting the surface layer with the first chemiluminescent substrate composition because Bronstein et al. teaches that the enhancer may be successfully added at any point in a methods for chemiluminescent detection of multiple substrates, such as those of Cheek et al. and Akhavan-Tafti '979. It would have been further obvious to employ polyvinylbenzyltrimethylammonium chloride or an onium copolymer

because Bronstein et al. teach that these are enhancers capable of significantly increasing the intensity of the chemiluminescent signal emitted in chemiluminescent detection methods. One would have a reasonable expectation of success because onium polymeric salts are taught as enhancers by Akhavan-Tafti (column 10, lines 26-47). It would have been further obvious to include BSA as an additive because Bronstein et al. teach that both BSA and onium polymers serve to enhance the chemiluminescent signal.

29. Claims 16-19 and 45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979, or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Wang et al. (WO 01/73134 A2).

Akhavan-Tafti '979, Akhavan-Tafti et al., and Cheek et al. are as discussed above, which fail to teach methods wherein the target molecules are pools of nucleic acids, comprise mRNA transcripts, cDNA or cRNA transcripts, or wherein the concentration of target nucleic acids is proportional to the expression level of genes. The references also fail to specifically teach cDNA target molecules labeled with digoxigenin (Akhavan-Tafti '979 does teach the digoxigenin-antidigoxigenin labeling system for mRNA; column 6, lines 25-34). However, the references fail to specifically teach a method wherein cDNA target molecules are labeled with digoxigenin.

Wang et al. teach ordered arrays of pools of target molecules (nucleic acids) on a solid support, where the mixtures reflect the expression profile of different cells or tissues (the abstract). Target molecules include mRNA or DNA derived from mRNA (such as cDNA), which can be used in microarray methods for analysis of gene expression in order to provide a relatively accurate indication of the level of expression of each gene in a cell (p. 24, lines 21-37

and p. 26, lines 24-33) Wang et al. also note that cDNA can be used in place of mRNA target samples because cDNA is more stable. Detecting of such targets can be used for mutation detection, genotyping, and DNA sequence analysis (p. 1, lines 10-15).

Therefore, it would have been obvious to one of ordinary skill in the art to employ the method of Cheek et al. and Akhavan-Tafti '979 (or Akhavan-Tafti '979 and Akhavan-Tafti) in order to detect pools of nucleic acids (such as mRNA, cDNA or cRNA transcripts) in order to analyze gene expression. One would have reasonable expectation of success because Wang et al. teach that such target molecules may be detected in microarray formats (such as that of Cheek et al.) using chemiluminescent detection (p. 3, line 35 to p. 4, line 2).

30. Claims 22-24 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979, or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Ferea et al. (US Patent 6,905,826 B2).

The references fail to specifically teach a method wherein control probes are located on the support surface, or wherein control probes are co-located in one or more of the same discrete areas as the plurality of probes.

Ferea et al. teach methods for detecting of target molecules in a sample using nucleic acid microarrays and in particular controls signals to be used in such methods. Such control signals allow for correction of irregularities in the shape, size, and intensity of microarray features (column 5, lines 49-52). Control signals additionally may be used to quantify the experimental signal (column 6, lines 16-19). Control oligonucleotide probes deposited on the array in the same discrete areas ("features") as the experimental probes can be used as hybridization controls (see

column 6, lines 41-60; claim 1 and Figure 4 in particular). The control labels, which may include fluorescent labels, to the surface of the array, which are used to confirm that the experimental probes are present and to help quantitate the experimental signal (column 6, lines 15-19 and claim 15). The relative amount of multiple experimental target sequences can be calculated by comparing the ratios of intensities of the experimental and control label signals. As one example, the control labels can be attached to the discrete area ("feature") by being conjugated to a probe (column 6, lines 41-60), which would be construed as a form of indirect attachment to the surface layer. They may also be attached covalently to the substrate, which would be construed as direct attachment.

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the control probes taught by Ferea et al. in the methods of Cheek et al. and Akhavan-Tafti '979 or Akhavan-Tafti '979 and Akhavan-Tafti et al. because Ferea et al. teach the benefit of control probes in determining whether hybridization is occurring in a microarray-format nucleic acid hybridization method and to help quantify the experimental signal. It would have been further obvious to include a fluorescent label as a control label and to compare the intensity of the signal from the fluorescent label to the experimental chemiluminescent signals because Ferea et al. teach that such control labels may be used to help quantify experimental signals in a microarray-format nucleic acid hybridization method using chemiluminescent and/or fluorescent detection. One would have a reasonable expectation of success because Ferea et al. teach that the control labels can used in conjunction with chemiluminescence labeling (see claim 17 in particular), which is the detection method of Cheek et al., Akhavan-Tafti '979, and Akhavan-Tafti.

31. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Gambini et al. (US Patent No. 6,518,068 B1).

The references fail to specifically teach a method wherein detection of the second chemiluminescent signal comprises filtering the emissions with a filter adapted to reduce the intensity of the first chemiluminescent signal relative to the intensity of the second.

Gambini et al. teach a detection workstation for analysis of luminescent signals that comprises a filter (or filters on a filter wheel) which permits the selection of different wavelength ranges, and which may be used to separate the emissions of different reagents emitting at different wavelengths. The workstation may be used in a method for detecting multiple luminescent signals emitting at different wavelengths (see the abstract and column 6, line 55 to column 7, line 13). Gambini et al. further teach that signals from multiple reagents are separated using the filters, which are designed to maximize the sensitivity of the target reagent emission, while minimizing the sensitivity to other non-target reagent emission (column 13, line 60 to column 14, line 10).

Therefore, it would have been obvious to one of ordinary skill in the art to employ the filter detection method taught by Gambini et al. in the methods of Cheek et al. and Akhavan-Tafti '979 or Akhavan-Tafti '979 and Akhavan-Tafti et al. because Gambini et al. teach that such filters may be used to separate signals at different wavelengths by multiple reagents in a method for detection of multiple luminescent signals.

32. Claims 34 and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 as applied to claim 1 above, and further in view of Bronstein et al. (US Patent No. 4,931,223). The references fail to specifically teach a method wherein the two enzymes are beta-galactosidase and alkaline phosphatase.

Bronstein et al. teach methods of chemiluminescent detection for detection of multiple analytes in a sample, employing two enzyme conjugates that are conjugates beta-galactosidase and alkaline phosphatase conjugates (column 8, lines 1-21). Bronstein et al. further teach a number of substrates that can be cleaved by these two enzymes, in particular 1,2-dioxetane substrates (see columns 3-7). The two enzymes can be used as labels to detect chemiluminescent emissions on a solid support (membrane) (Examples 1-2).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to substitute beta-galactosidase (and a corresponding 1,2-dioxetane substrate capable of being cleaved by this enzyme) as taught by Bronstein et al. for the horseradish peroxidase enzyme used by Cheek et al. in the method of Cheek et al. and Akhavan-Tafti et al. because Bronstein et al. teach that beta-galactosidase can be used for the same purpose as an enzyme label for use in conjunction with alkaline phosphatase in a two-enzyme chemiluminescence method for detection of multiple analytes in a sample, such as that of Cheek et al. One would have a reasonable expectation of success because Bronstein et al. teach that beta-galactosidase can be used to detect emissions from a solid support.

33. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 and Bronstein et al., or, alternatively, Akhavan-Tafti '979 in view of

Akhavan-Tafti et al. as applied to claim 34 above, and further in view of Voyta et al. (US Patent No. 5,145,772).

The references fail to specifically teach that the composition comprising a chemiluminescent substrate capable of being activated by alkaline phosphatase comprises a 0.1M solution of aminomethylpropanol and 1 mM $MgCl_2$ at a pH of 9.5.

Voyta et al. teach a chemiluminescence detection method employing alkaline phosphatase as an enzyme label, wherein the enzyme is used in a solution containing 0.05M carbonate or Tris buffer solution and 1 mM $MgCl_2$ at pH=9.5 (column 11, lines 49-55 and column 13, lines 10-17). The above solution is used in order to dissolve enhancer substances, which stabilize chemiluminescent substrates and thereby increase the intensity of chemiluminescent emissions (see also column 2, lines 45-65 and Table 2).

Although Voyta et al. teach carbonate rather than sodium phosphate, it is well known in the art that these buffers may be interchanged in order to maintain a solution pH of 9.5. Further, while the concentration of the buffer used by Voyta et al. differs, such a difference will generally not support patentability in most cases, constituting optimization of ranges within prior art conditions (see MPEP 2144.05).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a solution comprising a buffer with a buffering capacity sufficient to maintain a pH of 9.5 in conjunction with 1 mM $MgCl_2$ in the method of Cheek et al., Akhavan-Tafti '979 and Bronstein et al. (or Akhavan-Tafti '979 and Akhavan-Tafti) in order to dissolve enhancer substances and thereby enhance the chemiluminescent signal. One would have a

reasonable expectation of success because Voyta teaches that such a solution is effective in a chemiluminescence detection method employing alkaline phosphatase.

34. Claim 36 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 and Bronstein et al., or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claim 34 above, and further in view of Bobrow et al. (US Patent No. 5,196,306).

The references fail to specifically teach a method wherein the composition comprising a chemiluminescent substrate capable of being activated by beta-galactosidase is a 0.1M solution of sodium phosphate and 1 mM $MgCl_2$ at a pH of 7.0.

Bobrow et al. teach use of beta-galactosidase in a solution comprising 10 mM sodium phosphate, 1 mM $MgCl_2$ at pH 7.0 (column 17, paragraph 2). Beta-galactosidase stored in this solution was shown to be active (Figure 7).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a substrate solution containing 0.1M solution of sodium phosphate and 1 mM $MgCl_2$ at a pH of 7.0 because Bobrow et al. teach that a solution comprising sodium phosphate buffer and 1 mM $MgCl_2$ was an appropriate solution that would not destroy enzyme activity in an assay involving beta-galactosidase, such as that of Cheek et al. and Bronstein et al. Further, while the concentration of the buffer used by Voyta et al. differs, such a difference will generally not support patentability in most cases, constituting optimization of ranges within prior art conditions (see MPEP 2144.05).

35. Claims 37-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 as applied to claim 15 above, and further in view of Clothier (US Patent No. 6,852,503 B1). The references fail to specifically recite contacting a support surface with a composition comprising the first and second enzyme conjugates.

Clothier teaches a dual enzyme chemiluminescent substrate formulation for use in methods involving two enzymes. Clothier teaches combining the two chemiluminescent enzymes (horseradish peroxidase and alkaline phosphatase) together prior to contacting the enzymes with the well surface (column 6, lines 25-36).

With respect to claim 38, Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the enzyme conjugates of Cheek et al. and Akhavan-Tafti '979 together prior to contacting with the support surface since Clothier teaches that enzymes for chemiluminescent substrates may be successfully combined together in methods for chemiluminescence detection involving two enzymes, such as that of Cheek et al. and Akhavan-Tafti '979.

It would also have been obvious to one of ordinary skill in the art at the time of the invention to substitute the streptavidin-HRP conjugate of Cheek et al. with an antidigoxigenin-HRP conjugate in order to detect a second target molecule labeled with digoxigenin, because

Akhavan-Tafti '979 teaches that antigen-antibody and biotin-streptavidin are both examples of binding pairs that may be successfully used for the same purpose in a method of sequential chemiluminescent detection of multiple, differentially labeled target molecules such as that of Cheek et al.

36. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 as applied to claim 1 above, and further in view of Akhavan-Tafti et al. (US Patent No. 5,523,212).

Cheek et al. and Akhavan-Tafti '979 are as discussed above, which fails to specifically teach that the chemiluminescent quantum yield enhancing material further comprises counterion moieties listed in claim 43.

Akhavan-Tafti '212 teach chemiluminescent formulations for the detection of biological molecules that comprise enhancers and additives such as β-cyclodextrin, polyols, and sulfate (column 15, lines 45-59; column 16, lines 1-21; and Examples 13 and 20), and it is further taught that useful levels of light intensity compared to reagent background are obtained with reagents that incorporate dextran sulfate and β-cyclodextrin (Example 20).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ dextran sulfate and β-cyclodextrin, as taught by Akhavan-Tafti '212 in the chemiluminescent formulations comprising enhancers of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '212 teaches that such additives give rise to useful levels of light intensity over background in methods for chemiluminescent detection of biological molecules, such as those of Cheek et al., Akhavan-Tafti '979 and Akhavan-Tafti et al.

37. Claims 6 and 33-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claim 1 above, and further in view of Bronstein et al. (US Patent No. 4,931,223). Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to specifically teach a method wherein both the first and second chemiluminescent substrates are 1,2-dioxetane substrates or a method where the enzymes are β-galactosidase and alkaline phosphatase.

Bronstein et al. teach methods for detecting chemiluminescent emissions using two or more 1,2-dioxetane substrates, which may be used in quantifying several analytes when each of the 1,2-dioxetanes emits light of a different wavelength (column 2, line 42 to column 3, line 3; 6, lines 44-47; column 7, lines 5-19; column 8, lines 1-29 in particular). Bronstein et al. further teach that β-galactosidase and alkaline phosphatase may be used in the method for cleaving different cleavable dioxetane substituents, and that use of these enzymes to cleave 1,2-dioxetane substrates that emit light of different wavelengths enables multichannel assays to be performed (column 7, lines 5-19; column 13, "Assay Procedure").

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ two 1,2-dioxetane substrates in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. for the purpose of producing chemiluminescent signals because Bronstein et al. teach that two or more 1,2-dioxetane substrates may be successfully used in a chemiluminescent detection assay for the quantification of two or more analytes, such as that of Akhavan-Tafti and Akhavan-Tafti et al., in order to enable multichannel assays.

38. Claims 8 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claim 1 above, and further in view of Bronstein et al. (US Patent No. 6,602,658 B1).

Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein the surface layer is contacted with the enhancing material prior to contacting with the first chemiluminescent substrate composition.

Bronstein et al. teach a method of measuring gene activity using sequential chemiluminescent detection of signal from two or more chemiluminescent substrates, wherein chemiluminescent signal enhancers such as onium copolymers, polyvinylbenzyltrimethylammonium chloride or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide the quantum yield enhancing material before contacting the surface layer with the first chemiluminescent substrate composition, because Bronstein et al. teach that the enhancing material may be added at any point in a method of sequential detection of chemiluminescent signals such as that of Akhavan-Tafti '979. It would have been further obvious to employ onium copolymers, as taught by Bronstein et al. in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. in order to enhance the chemiluminescent signal.

39. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claim 1 above, and further in view of Fodor et al. (US 6,309,822 B1).

Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which teach methods of detecting chemiluminescent emissions on solid supports having probe densities of 100 per cm2, but which fail to specifically teach densities of 1,000 areas per cm2.

Fodor et al. teach high density probe arrays, in which greater than about 400,000 different probes can be immobilized per cm2 (see in particular the abstract; column 2, lines 33-43; column 3, lines 18-48). The high-density probe arrays can be used to detect and quantify target nucleic acid sequences and/or to monitor the expression of a multiplicity of genes (column 33, lines 20-31; column 5, lines 34-36; column 2, lines 53-61). Fodor et al. teach that the high density probe arrays offer several advantages, including reduced intra- and inter-array variability, increased information content, and higher signal-to-noise ratio (see column 12 to column 15, line 60). In particular, Fodor et al. note that the arrays have advantages over blotted arrays (which is the technique used in Bronstein et al.), such as significantly higher hybridization efficiencies (column 14, line 61 to column 15, line 12).

Therefore, it would have been obvious to one of ordinary skill in the art to employ the high density probe arrays of Fodor et al. as the solid support in the method of detecting chemiluminescent emissions of Akhavan-Tafti '979 and Akhavan-Tafti et al. in order to allow for increased information content and massively parallel processing of hybridization data, reduction of assay variability, and/or to detect and quantify a multiplicity of genes with increased information content and sensitivity.

One would have a reasonable expectation of success in using the solid support of Fodor et al. in the method of detecting chemiluminescent emissions of Akhavan-Tafti '979 and Akhavan-Tafti et al. because Fodor et al. teach that the microarrays may be used in methods employing chemiluminescent detection (column 49, lines 5-12, column 82, lines 43-65) and also that enzyme labels may be used (column 20, lines 51-61). One would also have a reasonable expectation of success because Fodor et al. teach that the solid support may be a nylon membrane (column 95, lines 49-57), which is the same material used as the solid support by Akhavan-Tafti et al.

40. Claim 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claim 1 above, and further in view of Greene et al. (US Patent No. 5,137,804).

Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to teach a method wherein the first substrate is a 1,2-dioxetane substrate and the second is an acridan, enol phosphate, or luminol substrate. Akhavan-Tafti '979 teaches that the first chemiluminescent substrate used in a sequential detection method must be capable of being inhibited, and that the use of a horseradish peroxidase substrate such as an acridan compound is preferred as the first chemiluminescent substrate because of the ability to inhibit peroxidase activity (column 7, lines 65-67 to column 8, lines 1-23).

Greene et al. teach inhibitors of the enzyme alkaline phosphatase, which include inorganic phosphate, chelating agents, and amino acids (column 6, lines 9-29), in the context of

enzyme-based detection methods using alkaline phosphatase, horseradish peroxidase, and other enzymes (column 6, lines 30-37 and column 10, lines 19-30).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a horseradish peroxidase substrate (such as an acridan) in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. as the second, rather than the substrate, and to use a 1,2-dioxetane substrate capable of being activated by alkaline phosphatase as the first, rather than the second substrate, for the same purpose in generating chemiluminescent signals because Greene et al. teaches that alkaline phosphatase, like horseradish peroxidase, may also be readily inhibited in assays that employ this enzyme, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

41. Claims 42-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claim 1 above, and further in view of Akhavan-Tafti (US Patent No. 5,523,212).

Akhavan-Tafti '979 and Akhavan-Tafti et al. teach preferred formulations for chemiluminescence but fails to specifically teach additives and counterions. Akhavan-Tafti '212 teach chemiluminescent formulations for the detection of biological molecules that comprise enhancers and additives such as β–cyclodextrin, polyols, and sulfate (column 15, lines 45-59; column 16, lines 1-21; and Examples 13 and 20), and it is further taught that useful levels of light intensity compared to reagent background are obtained with reagents that incorporate dextran sulfate and β–cyclodextrin (Example 20).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ dextran sulfate and β-cyclodextrin, as taught by Akhavan-Tafti '212 in the chemiluminescent formulations comprising enhancers of Akhavan-Tafti '979 and Akhavan-Tafti et al. in order to achieve useful light intensity over background in methods for chemiluminescent detection of biological molecules, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

Response to Arguments

42. Applicant's arguments filed 5/9/06 have been fully considered.

43. With respect to the rejections of claims 24 and 30 under 35 USC 112, 1st paragraph (new matter), Applicant argues that support may be found for "fluorescent labels attached directly or indirectly to the surface layer" may be found in the specification at page 15, lines 23-24 and at page 16, lines 9-11, to which the Examiner disagrees for the reasons set forth in the rejection above.

44. With respect to the rejections over Cheek et al. (see 35 USC 103(a) above), Applicant argues (p. 15-16) that the microchannel support of Cheek et al. is not a *two-dimensional* solid support surface layer. This argument is not persuasive because claim 1 recites a "**two-dimensional solid support surface layer**" and not a "**two-dimensional solid support**". The adjective "two-dimensional" modifies "solid support surface layer" and therefore refers to the surface layer of the solid support. This language does not require that the entire solid support be two-dimensional, only that the surface layer be so. Although the microchannel biochip of Cheek et al. is three-dimensional, the *surface layer* of the biochip is two-dimensional (see Figure 1).

45. With respect to the rejections over Akhavan-Tafti '979 in view of Akhavan-Tafti, Applicant's arguments that Akhavan-Tafti teaches away from the use of other substrates besides Lumigen APS substrates (see p. 16-18) have been considered but are not persuasive for reasons of record (see the previous Office action at p. 39-40). Applicant has selectively focused on the statement by Akhavan-Tafti that Lumigen APS was the "[k]ey to the success" without considering what this statement would imply to one of ordinary skill in the art *reading the reference as a whole*. The Examiner maintains that when read in context, the statement "[k]ey to the success of the method was the rapid detection at room temperature afforded by Lumigen APS" does not teach away from other substrates, but rather conveys to the skilled artisan the importance of using a substrate, such as one from the proprietary Lumigen APS family of substrates, that permits rapid detection at room temperature. As an author publishing on behalf of Lumigen, Inc., Akhavan-Tafti is simply pointing out that the proprietary Lumigen APS substrates have this desirable property.

Furthermore, the importance of using substrates that allow for rapid detection when detecting chemiluminescent emissions on solid supports was known to one of ordinary skill in the art, for example as evidenced by Cheek et al. at p. 5780, the first two paragraphs of the section "Results and Discussion". Moreover, Cheek et al. teach examples of substrates other than Lumigen APS that permit rapid detection, such that one skilled in the art at the time of the invention would recognize that there are substrates other than the proprietary substrate Lumigen APS that permit rapid detection.

Applicant further argues that Akhavan-Tafti, in teaching Lumigen APS (an alkaline phosphatase substrate), teaches away from use with the method of Akhavan-Tafti '979, which

employs a peroxidase substrate. As noted in the previous Office action, this argument is not persuasive since the method of Akhavan-Tafti '979 employs two substrates, one of which is specific for alkaline phosphatase and the second of which specific for "preferably alkaline phosphatase". Furthermore, the solid support material of Akhavan-Tafti is nylon membrane, which is the same material taught in Akhavan-Tafti '979. One skilled in the art would not be led away from the use of alkaline phosphatase substrates with the substrate of Akhavan-Tafti since Akhavan-Tafti '979 teach that nylon membrane is compatible with the use of alkaline phosphatase as well as peroxidase substrates.

46. The Declaration under 37 CFR 1.132 filed 6/1/06 is insufficient to overcome the rejections based upon Cheek et al., Akhavan-Tafti, or Akhavan-Tafti '979 as set forth in the previous Office action for the following reasons.

The Declaration has been submitted in order to demonstrate unexpected results or properties and states that significant improvements were seen in the presence of the chemiluminescent enhancing material TPQ.

The Declaration is ineffective to demonstrate unexpected results because a comparison of the closest prior art relied upon has not been made with the claimed invention. *In re Payne,* 203 USPQ 248; *In re Merchant,* 197 USPQ 7.85; *In re Lamberti,* 198 USPQ 278.

In the rejections made over Akhavan-Tafti '979 in view of Akhavan-Tafti, the closest prior art is Akhavan-Tafti '979. This reference also teaches the use of chemiluminescent enhancement materials in order to improve the signal/background ratio (see column 10, lines 27-47). Thus, the addition of a chemiluminescent enhancing material is not related to the features that distinguish the claimed invention from that of Akhavan-Tafti '979.

Furthermore, the Declaration is insufficient to demonstrate unexpected results because the property or result must actually be unexpected. *In re Skoll,* 187 USPQ 481, 484; *In re Coleman,* 205 USPQ 1172. In the instant case, the use of chemiluminescent enhancing materials was well known in the art for the purpose of improving chemiluminescent signal, for example as taught in Akhavan-tafti et al. Thus, the improvements seen with the use of TPQ would not actually be unexpected to one skilled in the art.

In addition, the Declaration is ineffective because the scope of the showing must be commensurate with the scope of the claims. *In re Coleman,* 205 USPQ 1172; *In re Greenfield,* 197 USPQ 227; *In re Lindener,* 173 USPQ 356; *In re Payne,* 203 USPQ 245. The Declaration relates to the effects of TPQ, while the claims do not recite this specific chemical but rather "a quantum yield enhancing material". In addition, the Declaration pertains to detection of chemiluminescent emissions on a film (see p. 2, item 6), while the claims recite a "two-dimensional solid support surface layer" that is not restricted to a film material.

In view of the foregoing, when all of the evidence is considered, the totality of the rebuttal evidence of nonobviousness fails to outweigh the evidence of obviousness.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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